[2] Directory of Restriction Endonucleases

By RICHARD J. ROBERTS

Table I is intended to serve as a directory to the restriction endonucleases that have now been characterized. In forming the list, all endonucleases that cleave DNA at a specific sequence have been considered restriction enzymes, although in most cases there is no direct genetic evidence for the presence of a host-controlled restriction–modification system.

Certain strains have been omitted from this list to save space. Thus the many different *Staphylococcus aureus* isolates containing an isoschizomer of *Sau*3A[1] are not listed individually. Similarly the numerous strains of gliding bacteria (orders *Myxobacterales* and *Cytophagales*) that showed evidence of specific endonucleases during a large-scale screening[2] are still rather poorly characterized.

Within Table I the source of each microorganism is given either as an individual or a national culture collection. The enzymes are named in accordance with the proposal of Smith and Nathans.[3] When two enzymes recognize the same sequence (i.e., are isoschizomers), the prototype (i.e., the first example isolated) is indicated in parentheses in column 3. The recognition sequences (column 4) are abbreviated so that only one strand, reading $5' \rightarrow 3'$, is indicated and the point of cleavage, when known, is indicated by an arrow ($\downarrow$). When two bases appear in parentheses, either one may appear at that position within the recognition sequence. Where known, the base modified by the corresponding methylase is indicated by an asterisk. $\mathring{A}$ is N^6-methyladenosine; $\mathring{C}$ is 5-methylcytosine. The frequency of cleavage (columns 5–8) has been experimentally determined for bacteriophage lambda (λ) and adenovirus-2 (Ad2) DNAs, but represents the computer-derived values from the published sequences of SV40[4] and ϕX174[5] DNAs. When more than one reference appears (column 9), the first contains the purification procedure for the restriction enzyme, the second concerns its recognition sequence, the third contains the purification procedure for the methylase,

1. E. E. Stobberingh, R. Schiphof, and J. S. Sussenbach, *J. Bacteriol.* **131**, 645 (1977).
2. H. Mayer and H. Reichenbach, *J. Bacteriol.* **136**, 708 (1978).
3. H. O. Smith and D. Nathans, *J. Mol. Biol.* **81**, 419 (1973).
4. V. B. Reddy, B. Thimmappaya, R. Dhar, K. N. Subramanian, B. S. Zain, J. Pan, P. K. Ghosh, M. L. Celma, and S. M. Weissman, *Science* **200**, 494 (1978).
5. F. Sanger, G. M. Air, B. G. Barrell, N. L. Brown, A. R. Coulson, J. C. Fiddes, C. A. Hutchison, III, P. M. Slocombe, and M. Smith, *Nature* (*London*) **265**, 687 (1977).

ISBN 0-12-181968-X

TABLE I

RESTRICTION ENDONUCLEASES

Microorganism	Source	Enzyme	Sequence	Number of cleavage sites: λ	Ad2	SV40	φX174	References[e]
Achromobacter immobilis	ATCC 15934	*Aim*I	?	?	?	?	?	6
Acinetobacter calcoaceticus	R. J. Roberts	*Acc*I	GT↓(A/C)(G/T)AC	7	8	1	3	7
	R. J. Roberts	*Acc*II (*Fnu*DII)	CGCG	>50	>50	0	14	7
Agrobacterium tumefaciens	ATCC 15955	*Atu*AI	?	>30	>30	?	?	8
Agrobacterium tumefaciens B6806	E. Nester	*Atu*BI (*Eco*RII)	CC(A/T)GG	>35	>35	16	2	9
Agrobacterium tumefaciens ID 135	C. Kado	*Atu*II (*Eco*RII)	CC(A/T)GG	>35	>35	16	2	10
Agrobacterium tumefaciens C58	E. Nester	*Atu*CI (*Bcl*I)	TGATCA	7	5	1	0	8
Anabaena catanula	CCAP 1403/1	*Aca*I	?	?	?	?	?	11
Anabaena cylindrica	A. deWaard	*Acy*I	GPu↓CGPyC	>14	>14	0	7	12
Anabaena subcylindrica	K. Murray	*Asu*I	G↓GNCC	>30	>30	11	2	11
Anabaena variabilis	K. Murray	*Ava*I	C↓PyCGPuG	8	?	0	1	13
	K. Murray	*Ava*II	G↓G(A/T)CC	>17	>30	6	1	13, 14 and 15
	K. Murray	*Ava*III	ATGCAT	?	?	3	0	16, 17 and 18
Anabaena variabilis [uw]	E. C. Rosenvold	*Avr*I (*Ava*I)	CPyCGPuG	8	?	0	1	19
	E. C. Rosenvold	*Avr*II	CCTAGG	1	2	2	0	19
Arthrobacter luteus	ATCC 21606	*Alu*I	AG↓CT	>50	>50	35	24	20
Arthrobacter pyridinolis	R. DiLauro	*Apy*I	CC(A/T)GG	>35	>35	16	2	21
Bacillus amyloliquefaciens F	ATCC 23350	*Bam*FI (*Bam*HI)	GGATCC	5	3	1	0	22
Bacillus amyloliquefaciens H	F. E. Young	*Bam*HI	G↓GATCC	5	3	1	0	23, 24
Bacillus amyloliquefaciens K	T. Kaneko	*Bam*KI (*Bam*HI)	GGATCC	5	3	1	0	22
Bacillus amyloliquefaciens N	T. Ando	*Bam*NI (*Bam*HI)	GGATCC	5	3	1	0	25
	T. Ando	*Bam*N_x	?	?	?	?	?	25 and 26
Bacillus brevis S	A. P. Zarubina	*Bbv*SI	GC(*T/A)GC	Specific methylase				27
	ATCC 9999	*Bbv*I	GC(T/)GC	>30	>30	23	14	28

Anabaena variabilis [uw]	E. C. Rosenvold	*Avr*I (*Ava*I)	CPyCGPuG	8	?	0	1	19
	E. C. Rosenvold	*Avr*II	CCTAGG	1	2	2	0	19
Arthrobacter luteus	ATCC 21606	*Alu*I	AG↓CT	>50	>50	35	24	20
Arthrobacter pyridinolis	R. DiLauro	*Apy*I	CC(A/T)GG	>35	>35	16	2	21
Bacillus amyloliquefaciens F	ATCC 23350	*Bam*FI (*Bam*HI)	GGATCC	5	3	1	0	22
Bacillus amyloliquefaciens H	F. E. Young	*Bam*HI	G↓GATCC	5	3	1	0	23, 24
Bacillus amyloliquefaciens K	T. Kaneko	*Bam*KI (*Bam*HI)	GGATCC	5	3	1	0	22
Bacillus amyloliquefaciens N	T. Ando	*Bam*NI (*Bam*HI)	GGATCC	5	3	1	0	25
	T. Ando	*Bam*N$_x$	?	?	?	?	?	25 and 26
Bacillus brevis S	A. P. Zarubina	*Bbv*SI	GC(*T/A)GC	Specific methylase				27
Bacillus brevis	ATCC 9999	*Bbv*I	GC(T/A)GC	>30	>30	23	14	28
Bacillus caldolyticus	A. Atkinson	*Bcl*I	T↓GATCA	7	5	1	0	29
Bacillus cereus	ATCC 14579	*Bce*14579	?	>10	?	?	?	22
Bacillus cereus	IAM 1229	*Bce*1229	?	>10	?	?	?	22
Bacillus cereus	T. Ando	*Bce*170 (*Pst*I)	CTGCAG	18	25	2	1	22
Bacillus cereus Rf sm st	T. Ando	*Bce*R (*Fnu*DII)	CGCG	>50	>50	0	14	22
Bacillus globigii	G. A. Wilson	*Bgl*I	GCCNNNN↓NGGC	22	12	1	0	30 and 31, 32
	G. A. Wilson	*Bgl*II	A↓GATCT	>6	12	0	0	30 and 31, 33
Bacillus megaterium 899	B899	*Bme*899	?	>5	?	?	?	22
Bacillus megaterium B205-3	T. Kaneko	*Bme*205	?	>10	?	?	?	22
Bacillus megaterium	J. Upcroft	*Bme*I	?	>10	>20	4	?	34
Bacillus pumilus AHU1387	T. Ando	*Bpu*1	?	6	>30	2	?	35
Bacillus sphaericus	IAM 1286	*Bsp*1286	?	?	?	?	?	22
Bacillus sphaericus R	P. Venetianer	*Bsp*RI (*Hae*III)	GGCC	>50	>50	19	11	36
Bacillus stearothermophilus 1503-4R	N. Welker	*Bst*I (*Bam*HI)	GGATCC	5	3	1	0	37
Bacillus stearothermophilus 240	A. Atkinson	*Bst*AI	?	?	?	?	?	38
Bacillus stearothermophilus ET	N. Welker	*Bst*EI	?	?	?	?	?	39
	N. Welker	*Bst*EII	?	11	8	0	0	39
	N. Welker	*Bst*EIII	?	>7	?	?	?	39
Bacillus subtilis strain X5	T. Trautner	*Bsu*RI (*Hae*III)	GG↓CC	>50	>50	19	11	40, 41, 42
Bacillus subtilis Marburg 168	T. Ando	*Bsu*M	?	>10	?	?	?	22
Bacillus subtilis	ATCC 6633	*Bsu*6663	?	>20	?	?	?	22
Bacillus subtilis	IAM 1076	*Bsu*1076 (*Hae*III)	GGCC	>50	>50	19	11	22

(*Continued*)

TABLE I—*Continued*

Microorganism	Source	Enzyme	Sequence	Number of cleavage sites				References[e]
				λ	Ad2	SV40	φX174	
Bacillus subtilis	IAM 1114	*Bsu*1114 (*Hae*III)	GGCC	>50	>50	19	11	22
Bacillus subtilis	IAM 1247	*Bsu*1247 (*Pst*I)	CTGCAG	18	25	2	1	22, 43
Bacillus subtilis	ATCC 14593	*Bsu*1145	?	>20	?	?	?	22
Bacillus subtilis	IAM 1192	*Bsu*1192	?	>10	?	?	?	22
Bacillus subtilis	IAM 1193	*Bsu*1193	?	>30	?	?	?	22
Bacillus subtilis	IAM 1231	*Bsu*1231	?	>20	?	?	?	22
Bacillus subtilis	IAM 1259	*Bsu*1259	?	>8	?	?	?	22
Bordetella bronchiseptica	ATCC 19395	*Bbr*I (*Hin*dIII)	AAGCTT	6	11	6	0	44
Brevibacterium albidum	ATCC 15831	*Bal*I	TGG↓CCA	15	17	0	0	45
Brevibacterium luteum	ATCC 15830	*Blu*I (*Xho*I)	C↓TCGAG	1	5	0	1	46
	ATCC 15830	*Blu*II (*Hae*III)	GGCC	>50	>50	19	11	47
Caryophanon latum L.	H. Mayer	*Cla*I	AT↓CGAT	12	?	0	0	48
Chloroflexus aurantiacus	A. Bingham	*Cau*I (*Ava*II)	GG(A/T)CC	>30	>30	6	1	49
		*Cau*II	?	>30	>30	0	?	49
Chromobacterium violaceum	ATCC 12472	*Cvi*I	?	?	?	?	?	6
Corynebacterium humiferum	ATCC 21108	*Chu*I (*Hin*dIII)	AAGCTT	6	11	6	0	6
	ATCC 21108	*Chu*II (*Hin*dII)	GTPyPuAC	34	>20	7	13	6
Corynebacterium petrophilum	ATCC 19080	*Cpe*I (*Bcl*I)	TGATCA	7	5	1	0	50
Diplococcus pneumoniae	S. Lacks	*Dpn*I	G*A↓TC	?	?	?	0	51, 52 and 53
Diplococcus pneumoniae	S. Lacks	*Dpn*II (*Mbo*I)	GATC	>50	>50	7	0	51, 52
Enterobacter cloacae	H. Hartmann	*Ecl*I	?	15	?	?	?	54
	H. Hartmann	*Ecl*II (*Eco*RII)	CC(A/T)GG	>35	>35	16	2	54
Enterobacter cloacae	DSM 30056	*Eca*I	G↓GTNACC	12	?	0	0	55
Escherichia coli RY13	R. N. Yoshimori	*Eco*RI	G↓A*ATTC	5	5	1	0	56, 57, 56, 58
	R. N. Yoshimori	*Eco*RI′	PuPuA↓TPyPy	>10	>10	24	16	59
Escherichia coli R245	R. N. Yoshimori	*Eco*RII	↓CC*(A/T)GG	>35	>35	16	2	60, 61 and 62, 60
Escherichia coli B	W. Arber	*Eco*B	TGA*(N)$_8$TGCT	?	?	?	?	63, 64 and 65, 66
Escherichia coli K	M. Meselson	*Eco*K	AAC(N)$_6$GTGC	?	?	?	?	67, 68, 69
Escherichia coli (PI)	K. Murray	*Eco*PI	AGACC	?	?	?	?	70, 71, 72 and 73, 74

Diplococcus pneumoniae	S. Lacks	*Dpn*I	GA↓TC	?	?	?	0	51, 52 and 53
Diplococcus pneumoniae	S. Lacks	*Dpn*II (*Mbo*I)	GATC	>50	>50	7	0	51, 52
Enterobacter cloacae	H. Hartmann	*Ecl*I	?	15	?	?	?	54
	H. Hartmann	*Ecl*II (*Eco*RII)	CC($^{A}_{T}$)GG	>35	>35	16	2	54
Enterobacter cloacae	DSM 30056	*Eca*I	G↓GTNACC	12	?	0	0	55

Escherichia coli RY13	R. N. Yoshimori	*Eco*RI	G↓A$\overset{*}{A}$TTC	5	5	1	0	56, 57, 56, 58
	R. N. Yoshimori	*Eco*RI′	PuPuA↓TPyPy	>10	>10	24	16	59
Escherichia coli R245	R. N. Yoshimori	*Eco*RII	↓CC($^{*A}_{T}$)GG	>35	>35	16	2	60, 61 and 62, 60
Escherichia coli B	W. Arber	*Eco*B	TG$\overset{*}{A}$(N)$_8$TGCT	?	?	?	?	63, 64 and 65, 66
Escherichia coli K	M. Meselson	*Eco*K	AAC(N)$_6$GTGC	?	?	?	?	67, 68, 69
Escherichia coli (PI)	K. Murray	*Eco*PI	AGACC	?	?	?	?	70, 71, 72 and 73, 74
Escherichia coli P15	W. Arber	*Eco*P15	?	?	?	?	?	75
Fusobacterium nucleatum A	M. Smith	*Fnu*AI (*Hin*fI)	G↓ANTC	>50	>50	10	21	76
	M. Smith	*Fnu*AII (*Mbo*I)	GATC	>50	>50	7	0	44
Fusobacterium nucleatum C	M. Smith	*Fnu*CI (*Mbo*I)	↓GATC	>50	>50	7	0	76
Fusobacterium nucleatum D	M. Smith	*Fnu*DI (*Hae*III)	GG↓CC	>50	>50	19	11	76
	M. Smith	*Fnu*DII	CG↓CG	>50	>50	0	14	76
	M. Smith	*Fnu*DIII (*Hha*I)	GCG↓C	>50	>50	2	18	76
Fusobacterium nucleatum E	M. Smith	*Fnu*EI (*Sau*3A)	↓GATC	>50	>50	7	0	76
Fusobacterium nucleatum 48	M. Smith	*Fnu*48 I	?	>50	?	?	?	76
Haemophilus aegyptius	ATCC 11116	*Hae*I	($^{A}_{T}$)GG↓CC($^{T}_{A}$)	?	?	11	6	77
	ATCC 11116	*Hae*II	PuGCGC↓Py	>30	>30	1	8	78, 79
	ATCC 11116	*Hae*III	GG↓C$\overset{*}{C}$	>50	>50	19	11	80, 41, 81
Haemophilus aphrophilus	ATCC 19415	*Hap*I	?	>30	?	?	?	44
	ATCC 19415	*Hap*II (*Hpa*II)	C↓CGG	>50	>50	1	5	82, 83
Haemophilus gallinarum	ATCC 14385	*Hha*I[a]	GACGC	>50	>50	0	14	82, 84 and 85
Haemophilus haemoglobinophilus	ATCC 19416	*Hhg*I (*Hae*III)	GGCC	>50	>50	19	11	44
Haemophilus haemolyticus	ATCC 10014	*Hga*I	G$\overset{*}{C}$G↓C	>50	>50	2	18	86, 86, 87
	ATCC 10014	*Hha*II (*Hin*fI)	GANTC	>50	>50	10	21	88
Haemophilus influenzae 1056	J. Stuy	*Hin*1056I (*Fnu*DII)	CGCG	>50	>50	0	14	89
	J. Stuy	*Hin*1056II	?	>30	>30	0	4	89

(*Continued*)

TABLE 1—*Continued*

Microorganism	Source	Enzyme	Sequence	Number of cleavage sites				References[e]
				λ	Ad2	SV40	φX174	
Haemophilus influenzae serotype b, 1076	J. Stuy	*Hin*bIII (*Hin*dIII)	AAGCTT	6	11	6	0	89
Haemophilus influenzae R_b	C. A. Hutchison	*Hin*bIII (*Hin*dIII)	AAGCTT	6	11	6	0	90 and 42
Haemophilus influenzae serotype c, 1160	J. Stuy	*Hin*cII (*Hin*dII)	GTPyPuAC	34	>20	7	13	89
Haemophilus influenzae serotype c, 1161	J. Stuy	*Hin*cII (*Hin*dII)	GTPyPuAC	34	>20	7	13	89
Haemophilus influenzae R_c	A. Landy, G. Leidy	*Hin*cII (*Hin*dII)	GTPyPuAC	34	>20	7	13	91
Haemophilus influenzae R_d (exo mutant)	S. H. Goodgal	*Hin*dI	C$\overset{*}{A}$C	Specific methylase				92, 93
	S. H. Goodgal	*Hin*dII	GTPy↓Pu$\overset{*}{A}$C	34	>20	7	13	94, 95, 92, 93
	S. H. Goodgal	*Hin*dIII	$\overset{*}{A}$↓AGCTT	6	11	6	0	96, 96, 92, 93
	S. H. Goodgal	*Hin*dIV	G$\overset{*}{A}$C	Specific methylase				92, 93
Haemophilus influenzae R_d 123	V. Tanyashin	*Hin*dGLU	?	?	?	?	?	97
Haemophilus influenzae R_f	C. A. Hutchison	*Hin*fI	G↓ANTC	>50	>50	10	21	90, 98 and 99
	C. A. Hutchison	*Hin*fII (*Hin*dIII)	AAGCTT	6	11	6	0	87
Haemophilus influenzae H-1	M. Takanami	*Hin*HI (*Hae*II)	PuGCGCPy	>30	>30	1	8	82
Haemophilus parahaemolyticus	C. A. Hutchison	*Hph*I[b]	GGTGA	>50	>50	4	9	90, 100
Haemophilus parainfluenzae	J. Setlow	*Hpa*I	GTT↓AAC	11	6	4	3	101, 102
	J. Setlow	*Hpa*II	C↓$\overset{*}{C}$GG	>50	>50	1	5	101, 102, 81
Haemophilus suis	ATCC 19417	*Hsu*I (*Hin*dIII)	A↓AGCTT	6	11	6	0	44
Herpetosiphon giganteus HP1023	J. H. Parish	*Hgi*AI	$G(^{T}_{A})GC(^{T}_{A})$↓C	20	?	0	3	103
Klebsiella pneumoniae OK8	J. Davies	*Kpn*I	GGTAC↓C	2	8	1	0	104, 105
Microcoleus species	D. Comb	*Mst*I	TGCGCA	>10	>15	0	1	106, 106a
Moraxella bovis	ATCC 10900	*Mbo*I	↓GATC	>50	>50	7	0	107
	ATCC 10900	*Mbo*II[c]	GAAGA	>50	>50	15	11	107, 108 and 109
Moraxella glueidi LG1	J. Davies	*Mgl*I	?	?	?	?	?	104
Moraxella glueidi LG2	J. Davies	*Mgl*II	?	?	?	?	?	104
Moraxella nonliquefaciens	ATCC 19975	*Mno*I (*Hpa*II)	C↓CGG	>50	>50	1	5	44, 110
	ATCC 19975	*Mno*II	?	>10	>6	2	?	44
Moraxella nonliquefaciens	ATCC 17953	*Mnl*I[d]	CCTC	>100	>100	52	35	111
Moraxella nonliquefaciens	ATCC 17954	*Mnn*I (*Hin*dII)	GTPyPuAC	34	>20	7	13	112
	ATCC 17954	*Mnn*II (*Hae*III)	GGCC	>50	>50	19	11	112

Haemophilus suis	ATCC 19417	*Hsu*I (*Hin*dIII)	A↓AGCTT	6				
Herpetosiphon giganteus HP1023	J. H. Parish	*Hgi*AI	$G(^{T}_{A})GC(^{T}_{A})\downarrow C$	20	?	0	3	103
Klebsiella pneumoniae OK8	J. Davies	*Kpn*I	GGTAC↓C	2	8	1	0	104, 105
Microcoleus species	D. Comb	*Mst*I	TGCGCA	>10	>15	0	1	106, 106a
Moraxella bovis	ATCC 10900	*Mbo*I	↓GATC	>50	>50	7	0	107
	ATCC 10900	*Mbo*II[c]	GAAGA	>50	>50	15	11	107, 108 and 109
Moraxella glueidi LG1	J. Davies	*Mgl*I	?	?	?	?	?	104
Moraxella glueidi LG2	J. Davies	*Mgl*II	?	?	?	?	?	104
Moraxella nonliquefaciens	ATCC 19975	*Mno*I (*Hpa*II)	C↓CGG	>50	>50	1	5	44, 110
	ATCC 19975	*Mno*II	?	>10	>6	2	?	44
Moraxella nonliquefaciens	ATCC 17953	*Mnl*I[d]	CCTC	>100	>100	52	35	111
Moraxella nonliquefaciens	ATCC 17954	*Mnn*I (*Hin*dII)	GTPyPuAC	34	>20	7	13	112
	ATCC 17954	*Mnn*II (*Hae*III)	GGCC	>50	>50	19	11	112
	ATCC 17954	*Mnn*III	?	>50	>50	?	?	112
	ATCC 17954	*Mnn*IV (*Hha*I)	GCGC	>50	>50	2	18	112
Moraxella osloensis	ATCC 19976	*Mos*I (*Mbo*I)	GATC	>50	>50	7	0	107
Moraxella species	R. J. Roberts	*Msp*I (*Hpa*II)	CCGG	>50	>50	1	5	113
Myxococcus virescens	H. Reichenbach	*Mvi*I	?	1	?	?	?	114
	H. Reichenbach	*Mvi*II	?	?	?	?	?	114
Neisseria gonorrhoea	G. Wilson	*Ngo*I (*Hae*II)	PuGCGCPy	>30	>30	1	8	115
Neisseria gonorrhoea	CDC 66	*Ngo*II (*Hae*III)	GGCC	>50	>50	19	11	116
Oerskovia xanthineolytica	R. Shekman	*Oxa*I (*Alu*I)	AGCT	>50	>50	35	24	117
	R. Shekman	*Oxa*II	?	?	?	?	?	117
Proteus vulgaris	ATCC 13315	*Pvu*I	CGATCG	4	7	0	0	28
	ATCC 13315	*Pvu*II	CAG↓CTG	15	22	3	0	28
Providencia alcalifaciens	ATCC 9886	*Pal*I (*Hae*III)	GGCC	>50	>50	19	11	34
Providencia stuartii 164	J. Davies	*Pst*I	CTGCA↓G	18	25	2	1	104, 118
Pseudomonas facilis	M. VanMontagu	*Pfa*I	?	>30	>30	?	?	47, 89
Rhodopseudomonas sphaeroides	R. Lascelles	*Rsp*I	?	3	12	0	?	119
Rhodopseudomonas sphaeroides	S. Kaplan	*Rsh*I (*Pvu*I)	CGATCG	4	7	0	0	120
Serratia marcescens S_b	C. Mulder	*Sma*I	CCC↓GGG	3	12	0	0	121, 122
Serratia species SA1	B. Torheim	*Ssp*I	?	?	?	?	?	123
Staphylococcus aureus 3A	E. E. Stobberingh	*Sau*3A (*Mbo*I)	GATC	>50	>50	7	0	124
Staphylococcus aureus PS96	E. E. Stobberingh	*Sau*96I (*Asu*I)	G↓GNCC	>30	>30	11	2	125

(*Continued*)

TABLE I—*Continued*

Microorganism	Source	Enzyme	Sequence	Number of cleavage sites				References[a]
				λ	Ad2	SV40	φX174	
Streptococcus faecalis subsp. *zymogenes*	R. Wu	*Sfa*I (*Hae*III)	GG↓CC	>50	>50	19	11	126
Streptococcus faecalis ND547	D. Clewell	*Sfa*NI	GATGC	>50	>30	6	12	8
Streptomyces achromogenes	ATCC 12767	*Sac*I	GAGCT↓C	2	7	0	0	127
	ATCC 12767	*Sac*II	CCGC↓GG	3	>25	0	1	127
	ATCC 12767	*Sac*III	?	>30	>30	?	?	127
Streptomyces albus	CM1 52766	*Sal*PI (*Pst*I)	CTGCAG	18	25	2	1	128
Streptomyces albus subsp. *pathociclicus*	KCC S0166	*Spa*I (*Xho*I)	CTCGAG	1	5	0	1	129
Streptomyces albus G	J. M. Ghuysen	*Sal*I	G↓TCGAC	2	3	0	0	130
	J. M. Ghuysen	*Sal*II	?	>30	?	?	?	130
Streptomyces bobiliae	ATCC 3310	*Sbo*I	?	?	?	?	?	131
Streptomyces bradiae	ATCC 3535	*Sbr*I	?	?	?	?	?	131
Streptomyces cupidosporus	KCC S0316	*Scu*I (*Xho*I)	CTCGAG	1	5	0	1	131
Streptomyces exfoliatus	H. Takahashi	*Sex*I (*Xho*I)	CTCGAG	1	6	0	1	129
Streptomyces goshikiensis	H. Takahashi	*Sgo*I (*Xho*I)	CTCGAG	1	6	0	1	129
Streptomyces griseus	ATCC 23345	*Sgr*I	?	0	7	0	?	127
Streptomyces hygroscopicus	?	*Shy*I	?	2	?	?	?	132
Streptomyces lavendulae	ATCC 8644	*Sla*I (*Xho*I)	C↓TCGAG	1	6	0	1	131
Streptomyces luteoreticuli	H. Takahashi	*Slu*I (*Xho*I)	CTCGAG	1	6	0	1	129
Streptomyces stanford	S. Goff, A. Rambach	*Sst*I (*Sac*I)	GAGCT↓C	2	7	0	0	133, 134
	S. Goff, A. Rambach	*Sst*II (*Sac*II)	CCGC↓GG	3	>25	0	1	133
	S. Goff, A. Rambach	*Sst*III (*Sac*III)	?	>30	>30	?	?	133
Thermoplasma acidophilum	D. Searcy	*Tha*I (*Fnu*DII)	CG↓CG	>50	>50	0	14	135
Thermopolyspora glauca	ATCC 15345	*Tgl*I (*Sac*II)	CCGCGG	3	>25	0	1	28
Thermus aquaticus YTI	J. I. Harris	*Taq*I	T↓CGA	>50	>50	1	10	136
	J. I. Harris	*Taq*II	?	>30	>30	4	6	44
Xanthomonas amaranthicola	ATCC 11645	*Xam*I (*Sal*I)	GTCGAC	2	3	0	0	130
Xanthomonas badrii	ATCC 11672	*Xba*I	T↓CTAGA	1	4	0	0	137
Xanthomonas holcicola	ATCC 13461	*Xho*I	C↓TCGAG	1	6	0	1	46
	ATCC 13461	*Xho*II	Pu↓GATCPy	>20	>20	3	0	89, 28

Streptomyces stanford	S. Goff, A. Rambach	*Sst*I (*Sac*I)	GAGCT↓C	2	7	0	0	133, 134
	S. Goff, A. Rambach	*Sst*II (*Sac*II)	CCGC↓GG	3	>25	0	1	133
	S. Goff, A. Rambach	*Sst*III (*Sac*III)	?	>30	>30	?	?	133
Thermoplasma acidophilum	D. Searcy	*Tha*I (*Fnu*DII)	CG↓CG	>50	>50	0	14	135
Thermopolyspora glauca	ATCC 15345	*Tgl*I (*Sac*II)	CCGCGG	3	>25	0	1	28
Thermus aquaticus YTI	J. I. Harris	*Taq*I	T↓CGA	>50	>50	1	10	136
	J. I. Harris	*Taq*II	?	>30	>30	4	6	44
Xanthomonas amaranthicola	ATCC 11645	*Xam*I (*Sal*I)	GTCGAC	2	3	0	0	130
Xanthomonas badrii	ATCC 11672	*Xba*I	T↓CTAGA	1	4	0	0	137
Xanthomonas holcicola	ATCC 13461	*Xho*I	C↓TCGAG	1	6	0	1	46
	ATCC 13461	*Xho*II	Pu↓GATCPy	>20	>20	3	0	89, 28
Xanthomonas malvacearum	ATCC 9924	*Xma*I	C↓CCGGG	3	12	0	0	122
	ATCC 9924	*Xma*II (*Pst*I)	CTGCAG	18	25	2	1	122
Xanthomonas nigromaculans	ATCC 23390	*Xni*I (*Pvu*I)	CGATCG	4	7	0	0	112
Xanthomonas oryzae	M. Ehrlich	*Xor*I (*Pst*I)	CTGCAG	18	25	2	1	138
	M. Ehrlich	*Xor*II (*Pvu*I)	CGATCG	4	7	0	0	138
Xanthomonas papavericola	ATCC 14180	*Xpa*I (*Xho*I)	C↓TCGAG	1	6	0	1	138

[a] *Hga*I cleaves as indicated: 5′ GACGCNNNNN↓ 3′
3′ CTGCGNNNNNNNNNN↓ 5′.

[b] *Hph*I cleaves as indicated: 5′ GGTGANNNNNNNN↓ 3′
3′ CCACTNNNNNNNN↑ 5′.

[c] *Mbo*II cleaves as indicated: 5′ GAAGANNNNNNNN↓ 3′
3′ CTTCTNNNNNNN↑ 5′.

[d] *Mnl*I cleaves 5 to 10 bases from the recognition sequence.

[e] Key to references:

6. S. A. Endow and R. J. Roberts, unpublished observations.
7. M. Zabeau and R. J. Roberts, unpublished observations.
8. D. Sciaky and R. J. Roberts, unpublished observations.
9. G. Roizes, M. Patillon, and A. Kovoor, *FEBS Lett.* **82,** 69 (1977).
10. J. M. LeBon, C. Kado, L. J. Rosenthal, and J. Chirikjian, *Proc. Natl. Acad. Sci. U.S.A.* **75,** 4097 (1978).
11. S. G. Hughes, T. Bruce, and K. Murray, unpublished observations.
12. A. DeWaard, J. Korsuize, C. P. van Beveren, and J. Maat, *FEBS Lett.* **96,** 106 (1978).

TABLE I—*Continued*

13. K. Murray, S. G. Hughes, J. S. Brown, and S. Bruce, *Biochem. J.* **159,** 317 (1976).
14. J. G. Sutcliffe and G. Church, unpublished observations.
15. C. Fuchs, E. C. Rosenvold, A. Honigman, and W. Szybalski, *Gene* **4,** 1 (1978).
16. G. Roizes, P.-C. Nardeux, and R. Monier, *FEBS Lett.* **104,** 39 (1979).
17. H. Shimatake and M. Rosenberg, unpublished observations.
18. K. Denniston-Thompson, D. D. Moore, K. E. Kruger, M. E. Furth, and F. R. Blattner, *Science* **198,** 1051 (1978).
19. E. C. Rosenvold and W. Szybalski, unpublished observations.
20. R. J. Roberts, P. A. Myers, A. Morrison, and K. Murray, *J. Mol. Biol.* **102,** 157 (1976).
21. R. DiLauro, unpublished observations.
22. T. Shibata, S. Ikawa, C. Kim, and T. Ando, *J. Bacteriol.* **128,** 473 (1976).
23. G. A. Wilson and F. E. Young, *J. Mol. Biol.* **97,** 123 (1975).
24. R. J. Roberts, G. A. Wilson, and F. E. Young, *Nature* (*London*) **265,** 82 (1977).
25. T. Shibata and T. Ando, *Biochim. Biophys. Acta* **442,** 184 (1976).
26. T. Shibata and T. Ando, *Mol. Gen. Genet.* **138,** 269 (1975).
27. B. F. Vanyushin and A. P. Dobritsa, *Biochim. Biophys. Acta* **407,** 61 (1975).
28. T. R. Gingeras and R. J. Roberts, unpublished observations.
29. A. H. A. Bingham, T. Atkinson, D. Sciaky, and R. J. Roberts, *Nucleic Acids Res.* **5,** 3457 (1978).
30. G. A. Wilson and F. E. Young, *in* "Microbiology 1976" (D. Schlessinger, ed.), p. 350. Am. Soc. Microbiol., Washington, D.C., 1976.
31. C. H. Duncan, G. A. Wilson, and F. E. Young, *J. Bacteriol.* **134,** 338 (1978).
32. T. Bickle, unpublished observations.
33. V. Pirrotta, *Nucleic Acids Res.* **3,** 1747 (1976).
34. R. E. Gelinas, P. A. Myers, and R. J. Roberts, unpublished observations.
35. S. Ikawa, T. Shibata, and T. Ando, *J. Biochem.* (*Tokyo*) **80,** 1457 (1976).
36. A. Kiss, B. Sain, E. Csordas-Toth, and P. Venetianer, *Gene* **1,** 323 (1977).
37. J. Catterall and N. Welker, *J. Bacteriol.* **129,** 1110 (1977).
38. A. H. A. Bingham, R. J. Sharp, and A. Atkinson, unpublished observations.
39. R. B. Meagher, unpublished observations.
40. S. Bron, K. Murray, and T. A. Trautner, *Mol. Gen. Genet.* **143,** 13 (1975).
41. S. Bron and K. Murray, *Mol. Gen. Genet.* **143,** 25–33 (1975).
42. U. Gunthert, M. Freund, and T. A. Trautner, *FEBS Symp.* **12,** (abstr.) (1978).
43. T. Hoshino, T. Uozumi, S. Horinouchi, A. Ozaki, T. Beppu, and K. Arima, *Biochim. Biophys. Acta* **479,** 367 (1977).
44. R. J. Roberts and P. A. Myers, unpublished observations.

45. R. E. Gelinas, P. A. Myers, G. A. Weiss, R. J. Roberts, and K. Murray, *J. Mol. Biol.* **114,** 433 (1977).
46. T. R. Gingeras, P. A. Myers, J. A. Olson, F. A. Hanberg, and R. J. Roberts, *J. Mol. Biol.* **118,** 113 (1978).
47. M. Van Montagu, unpublished observations.
48. H. Mayer, R. Grosschedl, H. Schutte, and G. Hobom, unpublished observations.
49. A. H. A. Bingham and J. Darbyshire, unpublished observations.
50. J. Fisherman, T. R. Gingeras, and R. J. Roberts, unpublished observations.
51. S. Lacks and B. Greenberg, *J. Biol. Chem.* **250,** 4060 (1975).

39. R. B. Meagher, unpublished observations
40. S. Bron, K. Murray, and T. A. Trautner, *Mol. Gen. Genet.* **143,** 13 (1975).
41. S. Bron and K. Murray, *Mol. Gen. Genet.* **143,** 25–33 (1975).
42. U. Gunthert, M. Freund, and T. A. Trautner, *FEBS Symp.* **12,** (abstr.) (1978).
43. T. Hoshino, T. Uozumi, S. Horinouchi, A. Ozaki, T. Beppu, and K. Arima, *Biochim. Biophys. Acta* **479,** 367 (1977).
44. R. J. Roberts and P. A. Myers, unpublished observations.
45. R. E. Gelinas, P. A. Myers, G. A. Weiss, R. J. Roberts, and K. Murray, *J. Mol. Biol.* **114,** 433 (1977).
46. T. R. Gingeras, P. A. Myers, J. A. Olson, F. A. Hanberg, and R. J. Roberts, *J. Mol. Biol.* **118,** 113 (1978).
47. M. Van Montagu, unpublished observations.
48. H. Mayer, R. Grosschedl, H. Schutte, and G. Hobom, unpublished observations.
49. A. H. A. Bingham and J. Darbyshire, unpublished observations.
50. J. Fisherman, T. R. Gingeras, and R. J. Roberts, unpublished observations.
51. S. Lacks and B. Greenberg, *J. Biol. Chem.* **250,** 4060 (1975).
52. S. Lacks and B. Greenberg, *J. Mol. Biol.* **114,** 153 (1977).
53. G. E. Geier and P. Modrich, *J. Biol. Chem.* **254,** 1408 (1979).
54. H. Hartmann and W. Goebel, *FEBS Lett.* **80,** 285 (1977).
55. H. Mayer, E. Schwarz, M. Melzer, and G. Hobom, unpublished observations.
56. P. J. Greene, M. C. Betlach, H. M. Goodman, and H. W. Boyer, *Methods Mol. Biol.* **7,** 87 (1974).
57. J. Hedgpeth, H. M. Goodman, and H. W. Boyer, *Proc. Natl. Acad. Sci. U.S.A.* **69,** 3448 (1972).
58. A. Dugaiczyk, J. Hedgpeth, H. W. Boyer, and H. M. Goodman, *Biochemistry* **13,** 503 (1974).
59. K. Murray, J. S. Brown, and S. A. Bruce, unpublished observations.
60. R. N. Yoshimori, Ph.D. Thesis, University of California, San Francisco (1971).
61. C. H. Bigger, K. Murray, and N. E. Murray, *Nature* (*London*), *New Biol.* **244,** 7 (1973).
62. H. W. Boyer, L. T. Chow, A. Dugaiczyk, J. Hedgpeth, and H. M. Goodman, *Nature* (*London*), *New Biol.* **244,** 40 (1973).
63. B. Eskin and S. Linn, *J. Biol. Chem.* **247,** 6183 (1972).
64. J. A. Lautenberger, N. C. Kan, D. Lackey, S. Linn, M. H. Edgell, and C. A. Hutchison, III, *Proc. Natl. Acad. Sci. U.S.A.* **75,** 2271 (1978).
65. J. V. Ravetch, K. Horiuchi, and N. D. Zinder, *Proc. Natl. Acad. Sci. U.S.A.* **75,** 2266 (1978).
66. J. A. Lautenberger and S. Linn, *J. Biol. Chem.* **247,** 6176 (1972).
67. M. Meselson and R. Yuan, *Nature* (*London*) **217,** 1110 (1968).
68. N. C. Kan, J. A. Lautenberger, M. H. Edgell, and C. A. Hutchison, III, *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **37,** 1499 (1978); also unpublished observations.
69. A. Haberman, J. Heywood, and M. Meselson, *Proc. Natl. Acad. Sci. U.S.A.* **69,** 3138 (1972).
70. A. Haberman, *J. Mol. Biol.* **89,** 545 (1974).
71. B. Bachi and V. Pirrotta, unpublished observations.
72. J. P. Brockes, *Biochem. J.* **133,** 629 (1973).
73. J. P. Brockes, P. R. Brown, and K. Murray, *Biochem. J.* **127,** 1 (1972).
74. J. P. Brockes, P. R. Brown, and K. Murray, *J. Mol. Biol.* **88,** 437 (1974).
75. J. Reiser and R. Yuan, *J. Biol. Chem.* **252,** 451 (1977).
76. A. Lui, B. C. McBride, and M. Smith, unpublished observations.
77. K. Murray, A. Morrison, H. W. Cooke, and R. J. Roberts, unpublished observations.

TABLE I—*Continued*

78. R. J. Roberts, J. B. Breitmeyer, N. F. Tabachnik, and P. A. Myers, *J. Mol. Biol.* **91,** 121 (1975).
79. C.-P.D. Tu, R. Roychoudhury, and R. Wu, *Biochem. Biophys. Res. Commun.* **72,** 355 (1976).
80. J. H. Middleton, M. H. Edgell, and C. A. Hutchison, III, *J. Virol.* **10,** 42 (1972).
81. M. B. Mann and H. O. Smith, *Nucleic Acids Res.* **4,** 4211 (1977).
82. M. Takanami, *Methods Mol. Biol.* **7,** 113 (1974).
83. H. Sugisaki and K. Takanami, *Nature (London), New Biol.* **246,** 138 (1973).
84. N. L. Brown and M. Smith, *Proc. Natl. Acad. Sci. U.S.A.* **74,** 3213 (1977).
85. H. Sugisaki, *Gene* **3,** 17 (1978).
86. R. J. Roberts, P. A. Myers, A. Morrison, and K. Murray, *J. Mol. Biol.* **103,** 199 (1976).
87. M. B. Mann and H. O. Smith, unpublished observations.
88. M. B. Mann, R. N. Rao, and H. O. Smith, *Gene* **3,** 97 (1978).
89. J. A. Olson, P. A. Myers, and R. J. Roberts, unpublished observations.
90. J. H. Middleton, P. V. Stankus, M. H. Edgell, and C. A. Hutchison, III, unpublished observations.
91. A. Landy, E. Ruedisueli, L. Robinson, C. Foeller, and W. Ross, *Biochemistry* **13,** 2134 (1974).
92. P. H. Roy and H. O. Smith, *J. Mol. Biol.* **81,** 427 (1973).
93. P. H. Roy and H. O. Smith, *J. Mol. Biol.* **81,** 445 (1973).
94. H. O. Smith and K. W. Wilcox, *J. Mol. Biol.* **51,** 379 (1970).
95. T. J. Kelly, Jr. and H. O. Smith, *J. Mol. Biol.* **51,** 393 (1970).
96. R. Old, K. Murray, and G. Roizes, *J. Mol. Biol.* **92,** 331 (1975).
97. V. I. Tanyashin, L. I. Li, I. O. Muizhnieks, and A. A. Baev, *Dokl. Akad. Nauk SSSR* **231,** 226 (1976).
98. C. A. Hutchison, III and B. G. Barrell, unpublished observations.
99. K. Murray and A. Morrison, unpublished observations.
100. D. Kleid, Z. Humayun, A. Jeffrey, and A. Ptashne, *Proc. Natl. Acad. Sci. U.S.A.* **73,** 293 (1976).
101. P. A. Sharp, B. Sugden, and J. Sambrook, *Biochemistry* **12,** 3055 (1973).
102. D. E. Garfin and H. M. Goodman, *Biochem. Biophys. Res. Commun.* **59,** 108 (1974).
103. N. L. Brown, M. McClelland, and P. R. Whitehead, unpublished observations.
104. D. L. Smith, F. R. Blattner, and J. Davies, *Nucleic Acids Res.* **3,** 343 (1976).
105. J. Tomassini, R. Roychoudhury, R. Wu, and R. J. Roberts, *Nucleic Acids Res.* **5,** 4055 (1978).
106. D. Comb, I. Schildkraut, and R. J. Roberts, unpublished observations.
106a. T. R. Gingeras, J. P. Milazzo, and R. J. Roberts, *Nucleic Acids Res.* **5,** 4105 (1978).

107. R. E. Gelinas, P. A. Myers, and R. J. Roberts, *J. Mol. Biol.* **114,** 169 (1977).
108. N. L. Brown, C. A. Hutchison, III, and M. Smith, *J. Mol. Biol.* (in press).
109. S. A. Endow, *J. Mol. Biol.* **114,** 441 (1977).
110. U. L. RajBhandary and B. Baumstark, unpublished observations.
111. M. Zabeau, R. Greene, P. A. Myers, and R. J. Roberts, unpublished observations.
112. F. Hanberg, P. A. Myers, and R. J. Roberts, unpublished observations.
113. M. Van Montagu, P. A. Myers, and R. J. Roberts, unpublished observations.
114. D. W. Morris and J. H. Parish, *Arch. Microbiol.* **108,** 227 (1976).

100. D. Kleid, Z. Humayun, A. Jeffrey, and A. Ptashne, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 293 (1976).
101. P. A. Sharp, B. Sugden, and J. Sambrook, *Biochemistry* **12**, 3055 (1973).
102. D. E. Garfin and H. M. Goodman, *Biochem. Biophys. Res. Commun.* **59**, 108 (1974).
103. N. L. Brown, M. McClelland, and P. R. Whitehead, unpublished observations.
104. D. L. Smith, F. R. Blattner, and J. Davies, *Nucleic Acids Res.* **3**, 343 (1976).
105. J. Tomassini, R. Roychoudhury, R. Wu, and R. J. Roberts, *Nucleic Acids Res.* **5**, 4055 (1978).
106. D. Comb, I. Schildkraut, and R. J. Roberts, unpublished observations.
106a. T. R. Gingeras, J. P. Milazzo, and R. J. Roberts, *Nucleic Acids Res.* **5**, 4105 (1978).

107. R. E. Gelinas, P. A. Myers, and R. J. Roberts, *J. Mol. Biol.* **114**, 169 (1977).
108. N. L. Brown, C. A. Hutchison, III, and M. Smith, *J. Mol. Biol.* (in press).
109. S. A. Endow, *J. Mol. Biol.* **114**, 441 (1977).
110. U. L. RajBhandary and B. Baumstark, unpublished observations.
111. M. Zabeau, R. Greene, P. A. Myers, and R. J. Roberts, unpublished observations.
112. F. Hanberg, P. A. Myers, and R. J. Roberts, unpublished observations.
113. M. Van Montagu, P. A. Myers, and R. J. Roberts, unpublished observations.
114. D. W. Morris and J. H. Parish, *Arch. Microbiol.* **108**, 227 (1976).
115. G. A. Wilson and F. E. Young, unpublished observations.
116. D. J. Clanton, J. M. Woodward, and R. V. Miller, *J. Bacteriol.* **135**, 270 (1978).
117. A. Stotz and P. Philippson, unpublished observations.
118. N. L. Brown and M. Smith, *FEBS Lett.* **65**, 284 (1976).
119. A. H. A. Bingham, A. Atkinson, and J. Darbyshire, unpublished observations.
120. J. Gardner and S. Kaplan, unpublished observations.
121. R. Greene and C. Mulder, unpublished observations.
122. S. A. Endow and R. J. Roberts, *J. Mol. Biol.* **112**, 521 (1977).
123. B. Torheim, personal communication.
124. J. S. Sussenbach, C. H. Monfoort, R. Schiphof, and E. E. Stobberingh, *Nucleic Acids Res.* **3**, 3193 (1976).
125. J. S. Sussenbach, P. H. Steenbergh, J. A. Rost, W. J. Van Leeuwen, and J. D. A. van Embden, *Nucleic Acids Res.* **5**, 1153 (1978).
126. R. Wu, C. T. King, and E. Jay, *Gene* **4**, 329 (1978).
127. J. R. Arrand, P. A. Myers, and R. J. Roberts, unpublished observations.
128. K. Chater, *Nucleic Acids Res.* **4**, 1989 (1977).
129. H. Shinotsu, H. Takahashi, and H. Saito, unpublished observations.
130. J. R. Arrand, P. A. Myers, and R. J. Roberts, *J. Mol. Biol.* **118**, 127 (1978).
131. H. Takahashi, M. Shimizu, H. Saito, Y. Ikeda, and H. Sugisaki, *Gene* **5**, 9 (1979).
132. F. Walter, M. Hartmann, and M. Roth, *FEBS Symp.* **12**, (abstr.) (1978).
133. S. Goff and A. Rambach, *Gene* **3**, 347 (1978); also unpublished observations.
134. F. Muller, S. Stoffel, and S. G. Clarkson, unpublished observations.
135. D. McConnell, D. Searcy, and J. G. Sutcliffe, *Nucleic Acids Res.* **5**, 1729 (1978).
136. S. Sato, C. A. Hutchison, III, and J. I. Harris, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 542 (1977).
137. B. S. Zain and R. J. Roberts, *J. Mol. Biol.* **115**, 249 (1977).
138. J. Shedlarski, M. Farber, and M. Ehrlich, unpublished observations.

TABLE II
LIST OF ENZYMES WITH KNOWN RECOGNITION SEQUENCES

Terminal extension	Restriction enzyme	Recognition sequence
Blunt ends	*Dpn*I	G$\overset{*}{A}$↓TC
	*Eco*RI′	PuPuA↓TPyPy
	*Sma*I	CCC↓GGG
	*Alu*I	AG↓CT
	*Pvu*II	CAG↓CTG
	*Fnu*DII	CG↓CG
	*Hae*I	(A/T)GG↓CC(A/T)
	*Hpa*I	GTT↓AAC
5′↓GATC	*Mbo*I	↓GATC
	*Bgl*II	A↓GATCT
	*Bam*HI	G↓GATCC
	*Bcl*I	T↓GATCA
	*Xho*II	Pu↓GATCPy
5′↓CG	*Hpa*II	C↓CGG
	*Taq*I	T↓CGA
	*Cla*I	AT↓CGAT
	*Acy*I	GPu↓CGPyC
5′↓TCGA	*Xho*I	C↓TCGAG
	*Sal*I	G↓TCGAC
5′↓AATT	*Eco*RI	G↓AATTC
5′↓AGCT	*Hin*dIII	A↓AGCTT
5′↓CCGG	*Xma*I	C↓CCGGG
5′↓CTAG	*Xba*I	T↓CTAGA
3′ TGCA↓	*Pst*I	CTGCA↓G
3′ GTAC↓	*Kpn*I	GGTAC↓C
3′ GC↓	*Sac*II	CCGC↓GG
3′ GCGC↓	*Hae*II	PuGCGC↓Py
3′ CG↓	*Hha*I	GCG↓C
3′ AGCT↓	*Sac*I	GAGCT↓C
5′ ↓CC(A/T)GG	*Eco*RII	↓CC(A/T)GG
5′ ↓GTNAC	*Eca*I	G↓GTNACC
5′ ↓NNNNN	*Hga*I	5′ ↓NNNNNNNNNNGCGTC 3′ 3′ ↑NNNNNCGCAG 5′
5′ ↓PyCGPu	*Ava*I	C↓PyCGPuG
5′ ↓ANT	*Hin*fI	G↓ANTC
5′ ↓GNC	*Asu*I	G↓GNCC
5′ ↓G(A/T)C	*Ava*II	G↓G(A/T)CC
5′ ↓(A/C)(G/T)	*Acc*I	GT↓(A/C)(G/T)AC
3′ N↓	*Hph*I	5′ GGTGANNNNNNNN↓ 3′ 3′ CCACTNNNNNNN↑ 5′
	*Mbo*II	5′ GAAGANNNNNNNN↓ 3′ 3′ CTTCTNNNNNNN↑ 5′

and the fourth describes its recognition sequence. In some cases two references appear in one of these categories when two independent groups have reached similar conclusions.

Table II contains a listing of enzymes for which the recognition sequence is known and which might be useful for preparing recombinant DNAs. They are grouped according to the nature of the fragment ends produced. Thus, fragments generated by all enzymes within any group can be joined to one another.

[3] Addition of Homopolymers to the 3′-Ends of Duplex DNA with Terminal Transferase[1]

By TIMOTHY NELSON and DOUGLAS BRUTLAG

The linkage of two DNAs *in vitro* to form recombinant molecules first became possible with the discovery of DNA ligases.[2] These enzymes, which seal nicks in DNA, can covalently join two DNAs that have complementary sticky ends such as the short, staggered ends generated by many restriction endonucleases.[3] Lobban and Kaiser[4] and Jackson *et al.*[5] showed that complementary ends could be added to DNA molecules *in vitro* with terminal transferase, thus allowing any two DNAs to be linked. These workers added complementary single-stranded homopolymers to two DNA molecules, annealed the homopolymer regions, and covalently closed the resulting hybrid *in vitro* with DNA polymerase I and DNA ligase from *Escherichia coli*. The DNA polymerase was necessary to trim any excess unpaired nucleotides at the 3′-ends or to fill in gaps generated by unequal lengths of the complementary homopolymer regions. Wensink *et al.*[6] simplified this procedure by showing that the annealed recombinant molecules were infectious and that they would be covalently closed *in vivo* during transfection.

Lobban and Kaiser[4] originally found that completely duplex molecules were inefficient primers for the terminal transferase reaction and that pretreatment of the DNA with lambda exonuclease to expose single-stranded

[1] This work was supported by a Basil O'Connor starter grant from the National Foundation-March of Dimes.

[2] I. R. Lehman, *Science* **186**, 790 (1974).

[3] J. E. Mertz and R. W. Davis, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 3370 (1972).

[4] P. E. Lobban and A. D. Kaiser, *J. Mol. Biol.* **78**, 453 (1973).

[5] D. A. Jackson, R. H. Symons, and P. Berg, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2904 (1972).

[6] P. C. Wensink, D. J. Finegan, J. E. Donelson, and D. S. Hogness, *Cell* **3**, 315 (1974).

ISBN 0-12-181968-X

Methods in Enzymology

Volume 68

Recombinant DNA

EDITED BY

Ray Wu

DIVISION OF BIOLOGICAL SCIENCES
SECTION OF BIOCHEMISTRY, MOLECULAR AND CELL BIOLOGY
CORNELL UNIVERSITY
ITHACA, NEW YORK

ACADEMIC PRESS, INC.
(Harcourt Brace Jovanovich, Publishers)
Orlando San Diego New York London
Toronto Montreal Sydney Tokyo

ACADEMIC PRESS, INC.
Orlando, Florida 32887

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1 7DX

Library of Congress Cataloging in Publication Data

Main entry under title:

Recombinant DNA.

(Methods in enzymology ; v. 68)
Bibliography: p.
Includes index.
1. Recombinant DNA. I. Wu, Ray.
II. Series.
QP601.M49 vol. 68 [QH442] 574.1'925'08s
ISBN 0-12-181968-X [574.8'732] 79-26584

PRINTED IN THE UNITED STATES OF AMERICA

85 86 87 88 9 8 7 6 5 4 3